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Experimental Gerontology 38 (2003) 491–498

Experimental Gerontology

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Altered methionine metabolism in long living Ames dwarf mice☆

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Received 20 November 2002; received in revised form 3 January 2003; accepted 9 January 2003

Abstract

Ames dwarf mice (df/df) are deficient in growth hormone, prolactin, and thyroid-stimulating hormone and live significantly longer than their normal siblings. In the current study, we found that the hormone deficiencies affect methionine metabolism. We previously reported that the dwarf mice exhibit enzyme activities and levels that combat oxidative stress more efficiently than those of normal mice. Moreover, methionine or metabolites of methionine are involved in antioxidative processes. Thus, we performed an experiment that compared various parameters of methionine metabolism between 18-month old male dwarf ($N = 6$) and wild type ($N = 5$) mice. The specific activity of liver methionine adenosyltransferase (MAT) was significantly elevated (205%, $p < 0.0001$) in the dwarf mice, as were cystathionine synthase (50%, $p < 0.01$), cystathionase (83%, $p < 0.001$), and glycine N-methyltransferase (GNMT, 91%, $p < 0.001$) activities. Even though the activities of MAT and GNMT were elevated, the concentration of liver *S*-adenosylmethionine was decreased (24%, $p < 0.001$) and *S*-adenosylhomocysteine increased (113%, $p < 0.001$) in the dwarf mice. These data indicate that dwarf mice, compared to wild type mice, have a markedly different metabolism of methionine. Altered methionine metabolism may partially explain earlier reports indicating less oxidative damage to proteins in dwarf mice. Taken together, the data suggest that methionine metabolism may play a role in oxidative defense in the dwarf mouse and should be studied as a potential mechanism of extended lifespan.

Keywords: Ames dwarf mouse; Long living; Methionine metabolism; *S*-adenosylmethionine; *S*-adenosylhomocysteine; Methionine adenosyltransferase; Glycine *N*-methyltransferase

1. Introduction

Long-lived Ames dwarf mice have a homozygous recessive mutation at the Prop-1 locus, which leads to a lack of differentiation of somatotrophic, lactotrophic, and thyrotrophic pituitary cells. As a result, these mice lack growth hormone (GH), prolactin, and thyroid stimulating hormone (Hauck et al., 2001). Recent studies by Brown-Borg (Brown-Borg et al., 1999; Brown-Borg and Rakoczy, 2000; Brown-Borg et al., 2001) have focused on the relevance of reduced GH signaling to longevity and lower oxidative stress in the dwarf mice. The significantly longer life span exhibited by these GH-deficient mice is contrasted with reduced life span and increased free radical processes in transgenic mice that overexpress GH (Rollo et al., 1996; Brown-Borg and Rakoczy, 2000). In further support of a role for GH in longevity determination, two additional mouse lines, the GH-receptor/binding protein knockout (Laron dwarf) mouse and a line of transgenic mice that overexpress a GH antagonist also live significantly longer than normal animals from the same strain (Bartke, 1998; Coschigano et al., 2000).

Brown-Borg reported that concentrations of hepatic inorganic peroxides and mitochondrial H_2O_2 were decreased and liver and kidney catalase were markedly

☆ Presented in part at American Aging Association (AGE) 2002, June 7-10, 2002, San Diego, CA [Uthus, E.O. and Brown-Borg, H.M (2002) Altered methionine metabolism in long living Ames dwarf mice. American Aging Association 31st Annual Meeting, p. 28]. The US Department of Agriculture, Agricultural Research Service, Northern Plains Area is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a trademark or proprietary product does not constitute a guarantee of warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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0531-5565/03/$ - see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0531-5565(03)00008-1

increased in Ames dwarf mice (Brown-Borg et al., 1999; Brown-Borg and Rakoczy, 2000; Brown-Borg et al., 2001). Catalase activity and protein were significantly elevated in livers from dwarf mice at 3, 6, 13–15, and 24 months of age when compared to age-matched wild type mice. Kidneys from old dwarf mice exhibited significantly increased catalase activity (22%), protein (16%) and mRNA expression (59%) compared to wild type mice (Brown-Borg and Rakoczy, 2000). Hypothalamic catalase has also been found to be elevated in these mice (Hauck and Bartke, 2000). The results of these studies suggested that GH status modulates antioxidative mechanisms and that catalase is important in overall defense capacity with respect to lifespan in the Ames dwarf mouse.

Growth hormone can also affect other metabolic pathways including methionine metabolism (Fig. 1). For example, glycine *N*-methyltransferase (GNMT), which plays a crucial role in the regulation of tissue concentrations of *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH), is regulated by GH (Aida et al., 1997).

Methionine metabolism also changes with aging. With age, the activities of Methionine adenosyltransferase (MAT), betaine homocysteine methyltransferase (BHMT), and methionine synthase decrease with age while cystathionine synthase (CS) and cystathionase (CTH) increase (Finkelstein, 1962; Finkelstein et al., 1971). MAT also decreases in male rats after puberty (Oscarsson et al., 2001). The concentration of SAM in the lens decreases with increasing age (Geller et al., 1988). The concentration of SAH in rat liver and cerebral cortex increases while brain SAM decreases with age (Hoffman et al., 1979; Trolin et al., 1994). Therefore, the expression of metabolites in the methionine metabolic pathway is tissue and age dependent.

As mentioned, the Ames dwarf mouse is lacking in GH, prolactin and thyroid stimulating hormone. These mice also have decreased concentrations of glucose, insulin, and thyroid hormones, and a reduced core body temperature (Borg et al., 1995; Hunter et al., 1999). Hypothyroid rats have decreased total plasma homocysteine and increased hepatic activities of CS and CTH; thyroid hormone

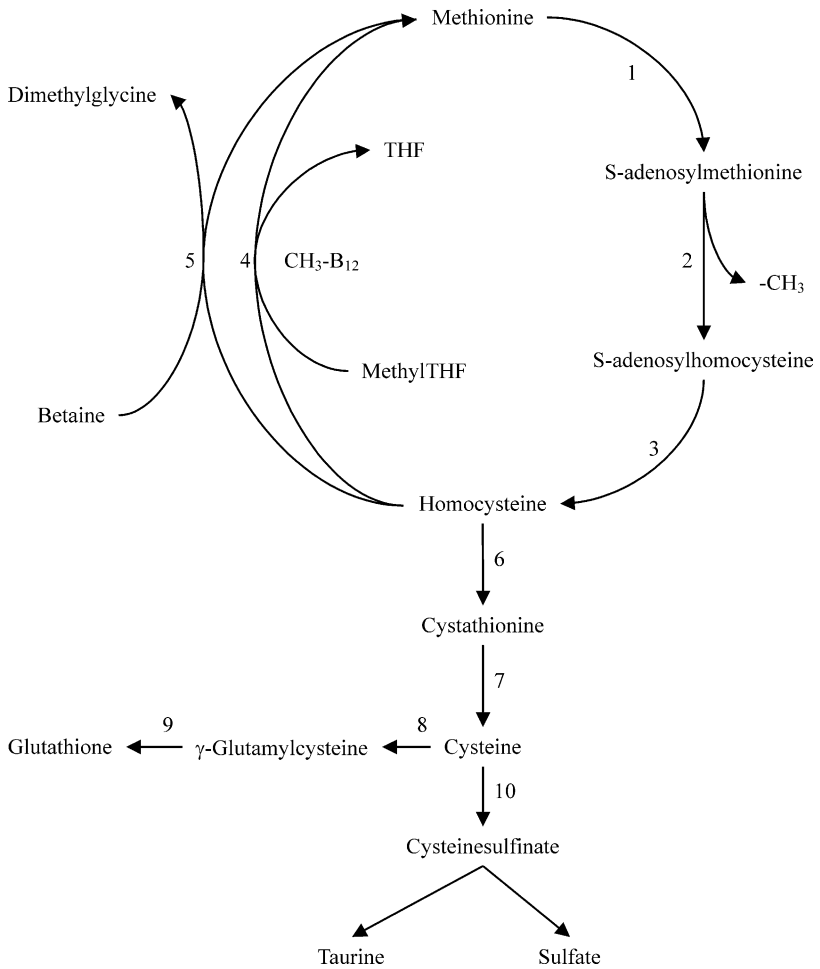

Fig. 1. Methionine metabolism. Enzymes: 1, MAT (*S*-adenosylmethionine synthase); 2, SAM-dependent transmethylases including glycine *N*-methyltransferase; 3, *S*-adenosylhomocysteine hydrolase; 4, Methionine synthase; 5, Betaine homocysteine methyltransferase; 6, CS; 7, γ-CTH; 8, γ-Glutamylcysteine synthetase; 9, Glutathione synthetase; 10, Cysteine dioxygenase.

administration to thyroidectomized rats normalized the plasma homocysteine and the activities of the enzymes (Jacobs et al., 2000). Pan and Tarver (Pan and Tarver, 1967) showed that thyroidectomized rats have increased activities of liver MAT and that administration of bovine GH to hypophysectomized rats resulted in a decrease in the activity of the enzyme. They also reported that administration of T_3 to normal rats resulted in a decreased activity of MAT. Keating et al. (Keating et al., 1988) also reported that the activity of MAT is elevated in hypothyroidism and that this resulted in an increased concentration of SAM.

Thus, the hormonal changes found in the Ames dwarf mouse may affect methionine metabolism. The objective of our studies was to determine whether or not methionine metabolism is indeed altered and if so, whether this may help explain the extended survival of these mice.

2. Materials and methods

2.1. Animals

Ames dwarf and age-matched wild type mice were maintained at the University of North Dakota (UND) vivarium facilities under controlled conditions of photoperiod (12 h light:12 h dark) and temperature (22 ± 1 °C) with free access to food (PMI Nutrition Intl., St. Louis, MO; Laboratory rodent diet) and water. The Ames dwarf (df/df) mice used in this study were derived from a closed colony with heterogeneous background (over 20 years). Dwarf mice were generated by mating either homozygous (df/df) or heterozygous (df/ +) dwarf males with carrier females (df/ +). Ames dwarf mice are maintained under standard laboratory conditions. All procedures involving animals were reviewed and approved by the UND Institutional Animal Care and Use Committee in accordance with the NIH guidelines for the care and use of laboratory animals. Liver and brain tissue from 18 month old animals ($N = 6$ dwarf, $N = 5$ normal, wild type) were collected, rapidly frozen and maintained at -80 °C until analysis.

2.2. Liver S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH)

Liver was homogenized in 5 volumes of cold 0.4 M perchloric acid and prepared for SAM and SAH analysis according to Davis et al. (Davis et al., 2000) and measured with a Dionex 4000i HPLC (Dionex Corp., Sunnyvale, CA) according to the procedure of Wagner et al. (Wagner et al., 1984).

2.3. Genomic DNA methylation

The methylation status of CpG sites in genomic DNA was determined by the in vitro methyl acceptance capacity of DNA by using [methyl-^{3}H]SAM as a methyl donor and prokaryotic CpG DNA methyltransferase, as described previously (Davis et al., 2000).

2.4. Liver enzyme activities

The activity of betaine-homocysteine methyltransferase (BHMT) was determined according to Finkelstein and Mudd (Finkelstein and Mudd, 1967) as modified by Xue and Snoswell (Xue and Snoswell, 1985). The substrate [methyl-^{3}H]betaine was prepared according to Xue and Snoswell (Xue and Snoswell, 1985). Liver was prepared by homogenization (1 g liver/4 ml buffer) in 0.03 M potassium phosphate buffer, pH 7.0. The homogenate was centrifuged at 36,000 × g for 10 min at 4 °C; the supernatant was used for the assay.

Methionine synthase (MS) activity was determined by the method of Sauer (Sauer, 1983). Liver was prepared as outlined for BHMT. MS was assayed in the presence of exogenous vitamin B_{12} in the reaction mixture.

The activity of CS was determined according to a modified method of Suda et al. (Suda et al., 1971) which is based on the method of Mudd et al. (Mudd et al., 1965). The reaction mixture of Suda et al. (Suda et al., 1971) was used but the reaction was stopped with 0.4 ml cold 10% TCA. A 0.5 ml aliquot was diluted to 20.5 and 0.01 ml 0.05 M cystathionine was added. The solution was applied to a Dowex 50-X4(H^+) (200–400 mesh) column, 0.9 × 3.0 cm. The column was washed with 18 ml H_2O, 35.5 ml 0.4N HCl, and then 12 ml H_2O; ^{14}C-labelled cystathionine product was then eluted with 6.0 ml 2N NH_4OH. Three ml of the eluant was counted for ^{14}C. Liver was prepared as outlined for BHMT.

The activity of CTH, (cystathionine γ-lyase) was determined by the method of Stipanuk (Stipanuk, 1979). For CTH, liver was prepared as outlined for BHMT.

Methionine adenosyltransferase (*S*-adenosylmethionine synthase, MAT) activity was determined by the method of Cantoni (Cantoni, 1955) with modifications. No glutathione was included in the reaction mix, KCl was added to a final concentration of 0.3 M and all volumes were reduced to one-half resulting in a final volume of 0.5 ml. The reaction was stopped with 0.5 ml cold 0.4 M perchloric acid. After centrifugation, the supernatant fluid was diluted with H_2O and analyzed for SAM by HPLC using the method of Bottiglieri (Bottiglieri, 1990). Liver was prepared as outlined for BHMT.

The activity of liver glycine *N*-methyltransferase (GNMT) was determined by the method of Cook and Wagner (Cook and Wagner, 1984). Liver was homogenized in 5 volumes of buffer as described by Cook et al. (Cook et al., 1989) and centrifuged at 100,000 × g for 60 min at 4 °C. The supernatant fluid was diluted prior to analysis (1 volume supernatant fluid/4 volume 0.01 M potassium phosphate buffer, pH 7.4).

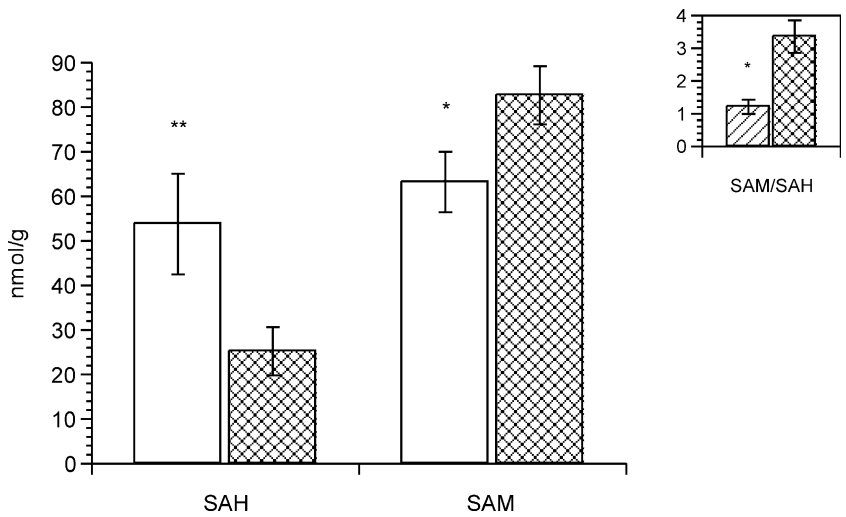

Fig. 2. Liver concentration of *S*-adenosylhomocysteine (SAH) and *S*-adenosylmethionine (SAM) (**, $p < 0.0006$; *, $p < 0.001$). Insert: ratio of liver SAM to SAH (*, $p < 0.0001$). Open bar-dwarf mice, hatched bar-wild type mice. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type.

2.5. Statistical analyses

Data analysis was performed using t tests for unpaired data to determine significance ($p \leq 0.05$) and presented as the mean ± standard deviation (SD) (Quattro Pro 8, Corel Corp., Ottawa, Ontario, Canada).

3. Results

As expected, body weights of dwarf mice were significantly lower when compared to the wild type mice (15.8 ± 2.3 vs 44.0 ± 4.8 g; $p < 0.0001$).

Liver SAM was significantly decreased (24%) and SAH considerably increased (113%) in the dwarf compared to wild type mice resulting in a marked decrease in SAM/SAH ratio (64%) in the dwarf (Fig. 2). *S*-adenosylhomocysteine was also increased (18%) in brain tissues of dwarf mice over that of wild type mice ($P < 0.02$; Fig. 3). However, brain SAM and the SAM/SAH ratio were unaffected by mouse type. DNA methylation in the liver of dwarf and wild type mice also did not appear to be different (Fig. 4).

When comparing Ames dwarf to wild type mice, the activities of several liver enzymes involved in methionine cycling and transsulfuration were altered (Figs. 5–8). Liver MAT activity was significantly elevated (205%) in the Ames dwarf compared to wild type mice (Fig. 5). Glycine *N*-methyltransferase, an enzyme that removes methyl groups from SAM, was also higher (91%) in liver tissues of dwarf mice over that of wild type mice ($p < 0.0003$; Fig. 6). Liver specific activity of CS was increased 50% ($p < 0.006$; Fig. 7) and CTH 83% ($p < 0.0006$; Fig. 8) in

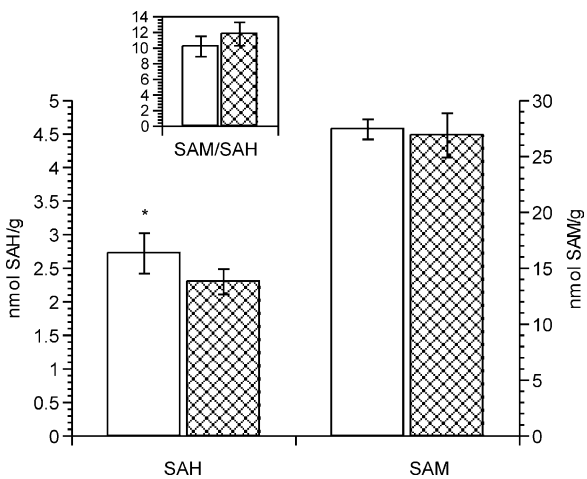

Fig. 3. Brain concentration of *S*-adenosylhomocysteine (SAH) and *S*-adenosylmethionine (SAM). Insert: ratio of liver SAM to SAH. Open bar-dwarf mice, hatched bar-wild type mice. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type.

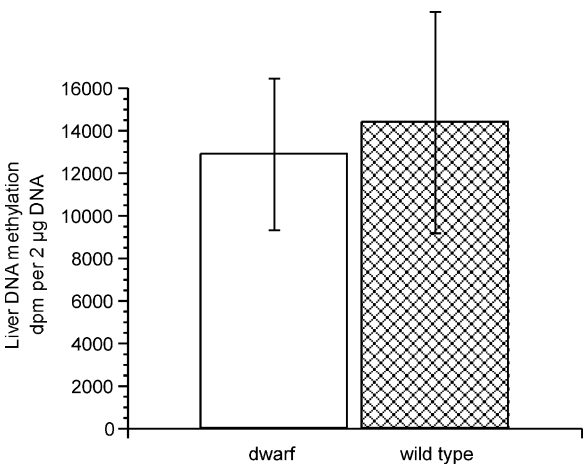

Fig. 4. Global DNA methylation status in liver. The extent of global DNA methylation is inversely proportional to the incorporation of methyl groups by bacterial *Sss* I methyltransferase in the presence of [^{3}H-methyl]SAM. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type.

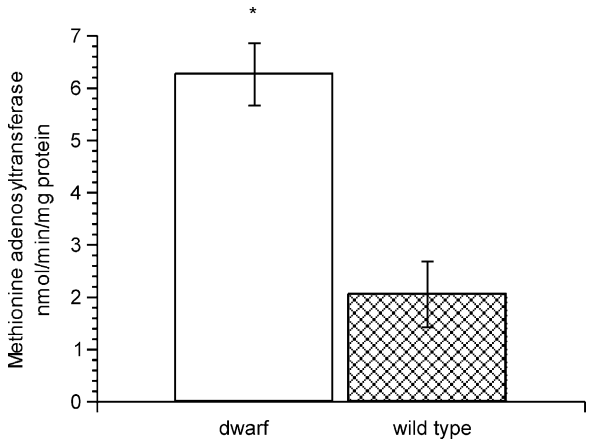

Fig. 5. Specific activity of liver methionine adenosyltransferase (MAT). Data are mean ± SD, $n = 6$ dwarf and 5 for wild type (*, $p < 0.0001$).

dwarf mice compared to age-matched normal mice. The activity of liver MS tended to be lower in the dwarf compared to the wild type mice ($p < 0.06$, Fig. 9). The activity of liver BHMT was not affected by mouse type ($p = 0.80$; Fig. 10).

4. Discussion

The results show that Ames dwarf mice, which live 49–64% longer than their normal counterparts, have a markedly altered methionine metabolism (Fig. 1). We found a significant increase in the specific activity of liver GNMT in the dwarf mice compared to wild type. Aida et al. (Aida et al., 1997) showed that hypophysectomized mice had high expression of GNMT and that treatment with GH decreased this expression; however, no mechanism for regulation of GNMT by GH was proposed. The Ames dwarf mice lack growth hormone as the result of a mutation in the Prop-1 locus. Thus, based on the findings of Aida et al. (Aida et al., 1997), it would be expected that the Ames dwarf mice would have an increased expression of GNMT. GNMT is thought to be an important regulator of tissue concentrations of SAM and SAH

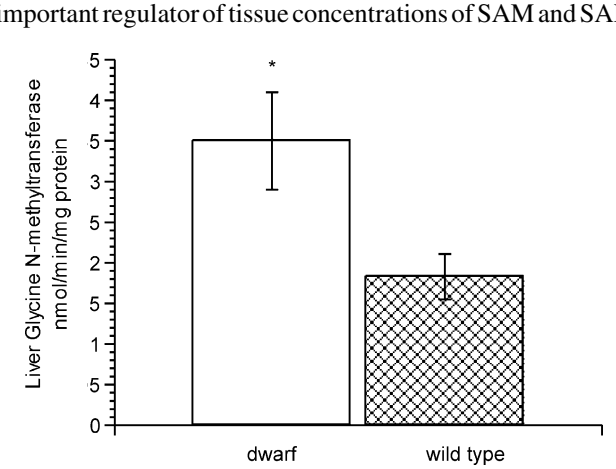

Fig. 6. Specific activity of liver glycine *N*-methyltransferase (GNMT). Data are mean ± SD, $n = 6$ dwarf and 5 for wild type (*, $p < 0.0001$).

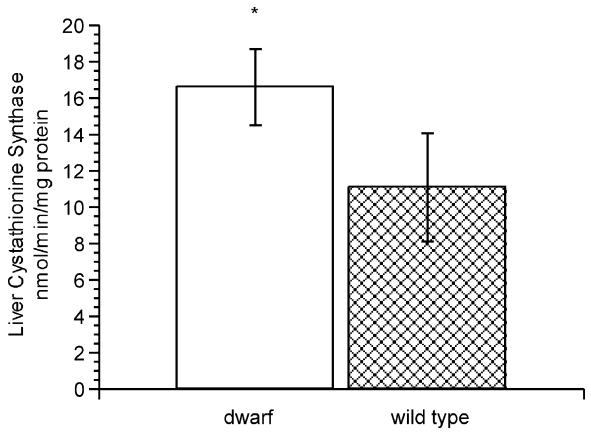

Fig. 7. Specific activity of liver CS. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type (*, $p < 0.006$).

and hence of SAM/SAH (Cook and Wagner, 1984; Wagner et al., 1985; Loehrer et al., 1996; Aida et al., 1997; Ogawa et al., 1998). Cook and Wagner (Cook and Wagner, 1984) reported that GNMT is a folate binding protein; 5MTHFpolyglutamate binds to and inhibits GNMT. SAM inhibits methylenetetrahydrofolate reductase (MTHFR) resulting in a decrease in the concentration of 5-MTHF polyglutamate. Thus, in normal animals, the activity of GNMT is increased in times of excess SAM. When SAM is low, there is no SAM-inhibition of MTHFR. This would result in increased inhibition of GNMT thereby conserving SAM for important biological methylations. This regulation, however, does not seem to occur in the dwarf mouse; the activity of GNMT remains high even though liver SAM is significantly decreased in the dwarf mouse. One possibility is that in the Ames dwarf mouse, the GH effect on GNMT is more prominent than the indirect regulation of GNMT by SAM. Also, GH and thyroid hormones can affect MAT. This, in combination with the effect of GH on GNMT, may explain some of the results including liver concentrations of SAM and SAH.

In this study, we found that dwarf mice exhibit a marked increase in the activity of liver MAT compared to wild type

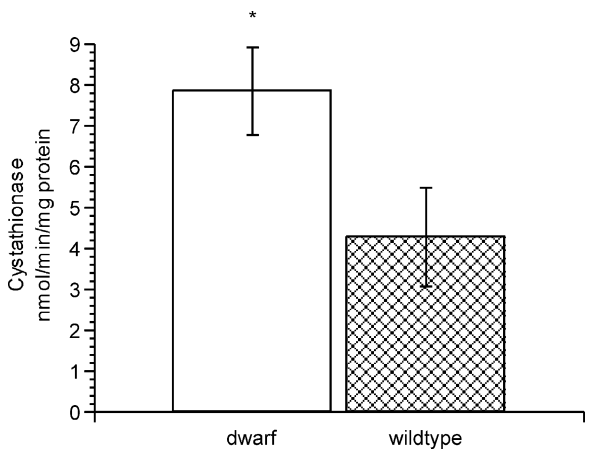

Fig. 8. Specific activity of liver CTH. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type (*, $p < 0.0006$).

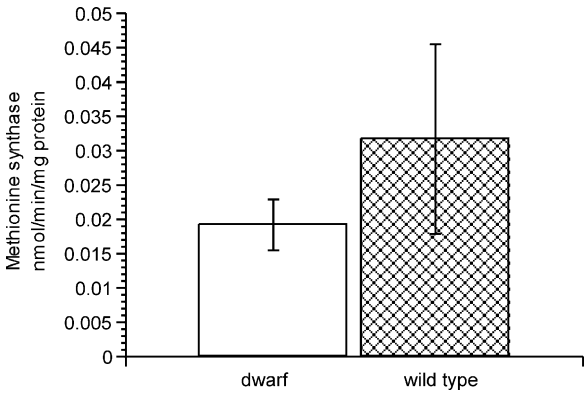

Fig. 9. Specific activity of liver MS. Data are mean ± SD, $n = 6$ dwarf and 5 for wild type ($p < 0.06$).

mice. Others have demonstrated that plasma hormone status affects MAT activity. Pan and Tarver (Pan and Tarver, 1967) showed that thyroidectomized rats have increased activities of liver MAT while the administration of T_3 to normal rats resulted in a decrease in the activity of MAT. In addition, the administration of bovine GH to hypophysectomized rats resulted in a decrease in the activity of the enzyme. Furthermore, Keating et al. (Keating et al., 1988) reported that the activity of MAT is elevated in hypothyroidism and that this resulted in an increased concentration of SAM. The Ames dwarf mice lack thyroid stimulating hormone as well as GH as the result of a mutation in the Prop-1 locus. Therefore, the increased activity of this enzyme was not unexpected based on earlier reports. In mammals, two genes, MAT1A and MAT2A, encode for MAT. MAT1A is expressed only in the liver as two isozymes (MAT III and MAT I). MAT2A is widely expressed and encodes for MAT II. MAT2A is also found in the fetal liver but is replaced by MAT1A during development. MAT II is inhibited by physiological concentrations of SAM. MAT III is thought to be important after a methionine load whereas MAT I and MAT II

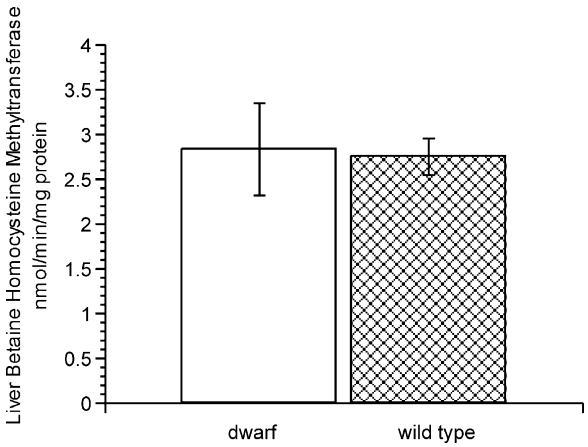

Fig. 10. Specific activity of liver betaine homocysteine methyltransferase (BHMT). Data are mean ± SD, $n = 6$ dwarf and 5 for wild type.

maintain basal levels of SAM (those required for liver under fasting conditions) (Carretero et al., 2001; Lu et al., 2001). The assay used to determine the activity of MAT in the current studies does not differentiate MAT I and III or, if expressed in the mouse livers, MAT II.

In our study, the hepatic concentration of SAM was decreased and SAH increased in the dwarf mice. This resulted in a significant decrease in SAM/SAH ratio. In normal animals an increase in SAH (or a decrease in SAM) resulting in a decreased ratio of SAM/SAH can be detrimental. A low SAM/SAH ratio can result in inhibition of SAM-dependent transmethylation reactions. Finkelstein (Finkelstein and Martin, 1986) reported that a SAM/SAH ratio of about 1.5 is consistent with inhibition of SAM-dependent transmethylation reactions. Another report cited Cantoni who calculated that when the SAM/SAH ratio drops to 1.6, there is a 20–80% inhibition of maximal activities of transmethylation reactions (Perna et al., 1993). However, the increased SAH and decreased SAM/SAH ratio do not seem to be detrimental to the dwarf mice. For example, we found no significant difference in global DNA methylation in dwarf mice compared to wild type. DNA methylation proceeds by SAM-dependent DNA methyltransferases. The incidence of spontaneous tumors in Ames dwarf mice is significantly lower and occurs much later than in wild type mice [Ikeno, personal communication]; (Bartke, 2000; Anisimov, 2001) and as already stated, the dwarf mice live significantly longer. Therefore, the decrease in liver SAM and increase in SAH in the dwarf mice must be viewed in relation to other findings. Also, the plasma concentration of homocysteine is decreased in dwarf mice compared to wild type (unpublished findings).

Although an isotope study is needed to truly determine the metabolic flux of methionine, we cannot rule out the possibility that the flux of methionine to the transsulfuration pathway is enhanced in the dwarf mice. We found that the activities of CS and CTH in dwarf mice compared to wild type are significantly increased. High activities of CS and CTH would indicate that more methionine (hence SAM) would be routed through the transsulfuration pathway. Because this pathway is irreversible, any methionine following the transsulfuration pathway is lost. We also show that the specific activities of liver BHMT and MS are not significantly different between the dwarf and wild type mice; MS tended to be decreased in the dwarf mice compared to the wild type. Also, the dwarf mice eat more food per g of body weight compared to the normal wild type mice (Mattison et al., 2000). Because of this and the increased activities of liver enzymes MAT, GNMT, CS, and CTH and the decreased concentration of liver SAM and concomitant increased concentration of SAH in dwarf mice, it seems plausible that more methionine is being routed through SAM and through the transsulfuration pathway. This is entirely different than a situation where methyl groups are limiting resulting in decreased SAM, increased SAH, fatty liver, hypomethylation, and carcinogenesis

(Wilson et al., 1984; Horne et al., 1989; Wainfan and Poirier, 1992).

In addition to increased oxidative defense, the long-lived Ames dwarf mouse appears to have an enhanced methionine metabolism. MAT, GNMT, CS, and CTH activities are increased in liver of the dwarf mice while the hepatic concentration of SAM is decreased and that of SAH increased. Because of the hormonal regulation of various enzymes in methionine metabolism, it is likely that the changes seen are the result of the mutation in the Prop-1 locus which results in the corresponding plasma GH, prolactin, and thyroid stimulating hormone deficiencies. While the significance of our findings is not yet known, it is possible that the changes in methionine metabolism could be a component of the long life, increased oxidative defense, and decrease in cancer associated with the Ames dwarf mouse.

Acknowledgements

The authors thank Kim Baurichter, Tom Zimmerman, Rhonda Poellot, and Sharlene Rakoczy for technical assistance and Cindy Davis for the DNA methylation work.

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